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Autobiographical sketch

DNA repair, DNA replication and human disorders: A personal journey

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I was born in 1946 and grew up in the industrial north-west of England close to the city of Manchester. My parents were German-Jewish refugees, who left Germany fairly early, in 1933. My father helped to establish and was one of the directors of a tannery, which made leather for shoes and handbags. This was part of a group of tanneries established first in Strasbourg by my great-grandfather Ferdinand Oppenheimer. I would describe my childhood and adolescent years as comfortable by general post-war standards. I went to a state primary school and obtained a scholarship to Manchester Grammar School (MGS), a fairly prestigious secondary school. As a child I was always interested in chemistry but had little interest in or knowledge of biology. The educational system in the UK at that time was such that one had to specialise very early and as a consequence I have had no formal biology education since the age of 12, something I have managed to hide reasonably successfully for the rest of my life! In my final two years at MGS I studied just physics, chemistry and mathematics and obtained a scholarship to Pembroke College, Cambridge (England) to study Natural Sciences, with the intention of becoming a chemist. In the second year at Cambridge, one of the options was a course on biochemistry. Having no real idea what this was, I read a book about it in the summer of 1965, and was truly astonished and excited to discover that the basis of life was just a bunch of rather complicated organic chemistry reactions. So I took the biochemistry course in my second year. By the end of that year, I was fed up with chemistry and for my final year I chose to do biochemistry rather than chemistry, a decision I have not regretted. The biochemistry lectures must have been pretty up-to-date, as we were told briefly about the discovery of DNA repair by Dick Setlow [1], a topic that seemed rather esoteric at the time.

1. PhD and postdoc: London, Tennessee and Sussex

In early 1967, in my final year at Cambridge, I saw an advertisement for a PhD position at the Chester Beatty Research Institute in London and went for an interview with Professor Peter Alexander, Christopher Dean and John Lett, a group of well-known radiobiologists. The work they proposed, to measure repair of double-strand breaks in mammalian cells, sounded interesting and I was, as it turned out erroneously, offered a place without any conditions. PhD positions are always offered on condition of obtaining a first or upper second class degree. Although I was expected to, and indeed went on to get a first-class degree, I decided that a bird in the hand was worth two in the bush and accepted the offer, another decision that I did not regret. I started my PhD studies in a new building in South London in October 1967 to discover that my proposed supervisor, John Lett, had taken a job in USA and his successor was a radiation physicist, Mike Ormerod. I was actually the only biochemist in the small department and this, together with my Cambridge degree, gave me a certain status above my station. Another important event that took place at the end of that year was my marriage, at what now seems like the ludicrously tender age of 21, to Judy Selbourne, a history graduate also from Manchester. This was a third decision I have not regretted and we have now been married for 43 years!

Measuring double-strand breaks was not easy. In 1967 McGrath and Williams had shown that single-strand breaks could be analysed by lysing bacterial cells on top of alkaline sucrose gradients and spinning out the denatured DNA strands in the ultracentrifuge [2]. The number of single-strand breaks could be measured from the rate of sedimentation of the DNA fragments. The Chester Beatty group had shown that this technique could also be applied to mammalian cells [3], and my job was to do similar measurements using neutral sucrose gradients to measure double-strand breaks. I was able to obtain a peak of fragmented DNA without difficulty. Unfortunately the position of the DNA remained the same, irrespective of the dose to which the cells were exposed, over a dose-range of 300–2000 Gy. In other words, it appeared that over a 10-fold dose range, there was no change in the apparent number of double-strand breaks. I struggled for a year trying to solve this mysterious phenomenon before temporarily abandoning the project.

1968 saw a great leap forward in the field of DNA replication with the discovery of Okazaki fragments in *Escherichia coli* [4], and this was followed by a similar claim for mammalian cells [5]. We realised however that the results obtained did not actually measure Okazaki fragments but were instead a consequence of a labelling artefact. This led to my first paper being a short note in Nature [6] (Fig. 1A) – the only first/last author paper I have managed to publish in Nature! It turned out of course that Okazaki fragments did indeed exist in mammalian cells, but the paper with the first claim was definitely wrong. We completed a mathematical analysis of this labelling artefact and were able to use it in a more positive way to measure the rate of replication fork progression in mouse cells [7]. At about that time, I came across a paper on centrifugation of high molecular weight DNA, showing that if DNA is centrifuged at high speeds, the rotor speed could affect sedimentation rates of DNA.

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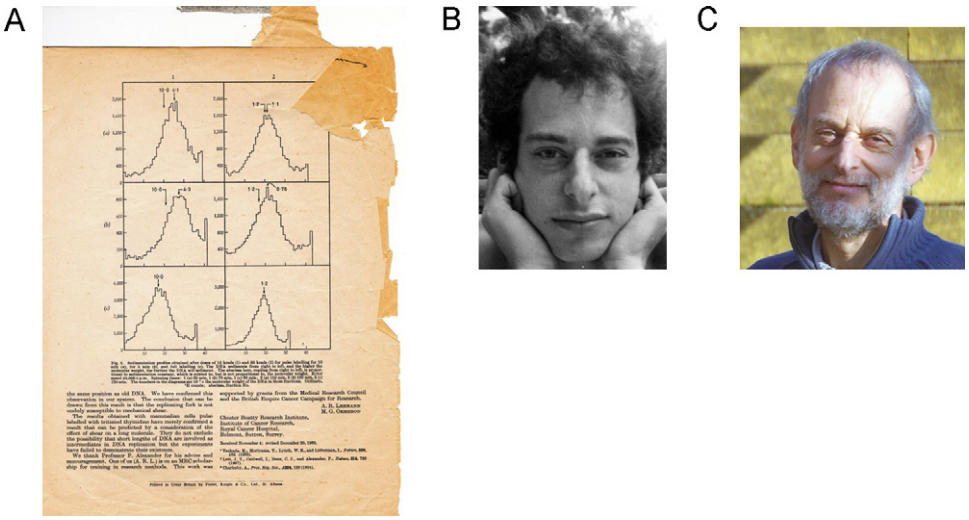

Fig. 1. (A) A page from my first paper, which, like the author, is showing signs of wear and tear forty years later (B, 1970; C, 2010).

I rushed back from the library, repeated my double-strand break experiments, but now ran the centrifuge at 10,000 rpm rather than 40,000 rpm and, look and behold, I obtained a nice dose–response for the induction of double-strand breaks and was able to show that this was linear for X-irradiation of mouse cells from 300–4000 Gy [8]. I did not detect any repair, most likely because I was using such high radiation doses. Unfortunately at lower doses, the DNA formed an aggregate and could not be analysed. My PhD work finished with an analysis of replicon sizes in mouse cells, again using alkaline sucrose gradients [9].

In the 1960–1970s getting one's BTA (Been To America) was de rigeur for a scientific career in the UK and Peter Alexander suggested I go to the lab of Dick Setlow, who had discovered excision-repair a few years earlier. Dick was kind enough to accept me for a postdoctoral position in his lab in Oak Ridge, Tennessee, in 1970. We arrived at Knoxville, Tennessee, airport in September 1970, laden with luggage and wearing our thick English overcoats so as not to fill up valuable luggage space. As the temperature was about 28 °C and the humidity close to 100%, Dick did not have too much trouble identifying us. Coming from London, arrival in Tennessee was something of a culture shock – we quickly learned that going more than 100 m on foot was considered by the locals to be close to insanity if you were able to travel by car. In the lab, things were also pretty different. I had been used to chatting to Mike Ormerod every day about my work during my PhD thesis, whereas Dick Setlow was very much hands-off. I floundered around for about 3 months searching for a decent project and getting a little despondent, before latching onto a topic that was to occupy much of my future career.

Dean Rupp and Paul Howard-Flanders had published their seminal work on postreplication repair in *E. coli* a couple of years earlier, in which they demonstrated daughter-strand gaps in UV-irradiated cells and showed that these gaps were subsequently sealed [10]. I thought it would be interesting to look if something similar happened in mammalian cells. Shortly after starting this project, I had the good fortune to attend the Biophysical Society meeting in New Orleans in February 1971, where I met Dean Rupp, Paul Howard-Flanders, Phil Hanawalt and other luminaries in the DNA repair field. I learned about the elegant experiments of Rupp and colleagues, in which they demonstrated that filling of daughter strand gaps in *E. coli* was effected by sister strand exchanges [11]. Dick Setlow had developed an elegant procedure for measuring gap sizes during nucleotide excision repair, by allowing the cells to incorporate bromodeoxyuridine (BrdU) into the gaps. BrdU-containing DNA is susceptible to breakage by UV light of 313 nm, so the filled-in gaps could be reconverted into breaks, and the size of the filled-in gap was inversely proportional to the dose of 313 nm irradiation needed to cleave the patch [12,13]. I realised that I could adapt this method to measure the size of daughter strand gaps during postreplication repair (PRR) and to determine whether the gaps were filled in by recombination with parental DNA, in which case they would not contain BrdU and would not be cleavable by 313 nm light. Alternatively if they were filled in by some kind of direct synthesis (that we now call translesion synthesis (TLS)) they would be cleavable. My results favoured the latter mechanism and argued against gap-filling by recombination in mammalian cells. To this day, I feel that this was the smartest experiment that I have done through my career, and I was excited to get it published in J. Mol. Biol. [14], at the time one of the top journals. (Even in those days, getting papers published in the best journals was important – I remember Mike Ormerod and I ranting about the stupid editor who had rejected one of our papers during my PhD. Le plus ça change!) Dick Setlow gave me the opportunity to present my data at the Gordon Conference on Nucleic Acids that he was chairing in the summer of 1971. I remember Paul Howard-Flanders giving me a hard time about interpretation of my data, but I got to meet another bunch of contemporary DNA repair stars such as Larry Grossman, and up and coming ones like Errol Friedberg and Priscilla Cooper.

My year in the US was highly formative and by the end of it, I had met almost everyone who was anyone in the DNA repair field and had generated data for two nice papers. That would certainly be extremely difficult nowadays. We returned to the UK in September 1971 and had to make cultural re-adjustments to small cars, public transport and gardens with roses. I had arranged a second postdoc at the University of Sussex with Sydney Shall. Sussex was one of a group of universities that were set up in the UK in the 1960s and it had established a good academic reputation in a few years as well as being regarded as pretty avant-garde. One reason I had chosen Sussex was that the professor of biochemistry, Asher Korner, had taught me about protein synthesis when I was an undergraduate at Cambridge and had particularly impressed me. I was distressed to discover on my arrival at Sussex that he had died very suddenly a few weeks earlier while only in his early 40s.

Sydney Shall was one of the first people to work on PARP, which at that time was a novel polymer of completely unknown function. For the next two years I dabbled a bit in studying PARP [15] as well as consolidating my work on PRR. I was singularly unimpressed and dismissive when Sydney suggested that PARP might be involved in

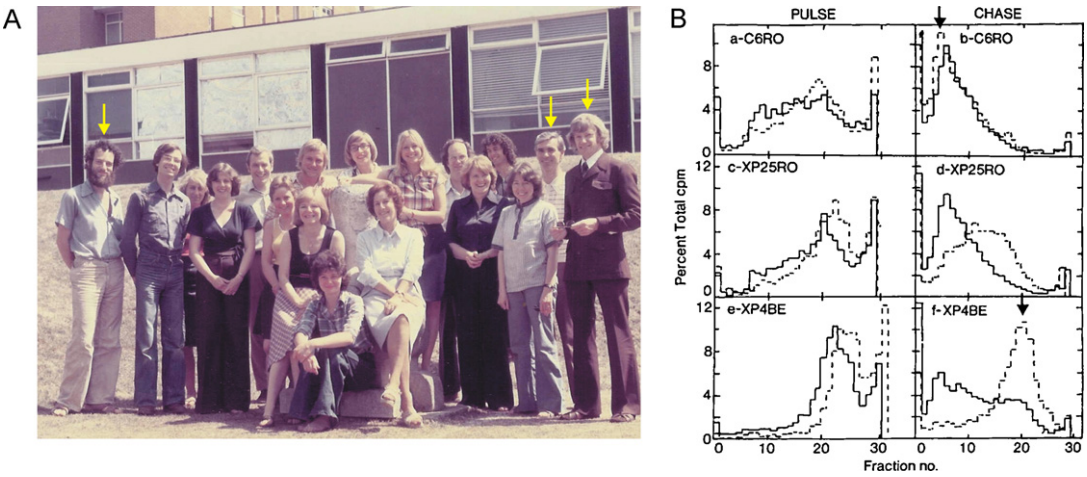

Fig. 2. 1975–1976. (A) Members of the CMU in 1976. *Note*: The shack which housed the labs, the author in more hirsute days (far left), Colin Arlett and Bryn Bridges (back row, right). (B) Figure from our 1975 PNAS paper, showing the dramatic difference in the size of newly synthesised DNA in UV-irradiated normal and XPV cells in the presence of caffeine (indicated by arrows).

DNA repair, which does not say much for my vision or instinct! After I had left his lab, he went on to show that PARP inhibitors do indeed sensitise cells to DNA damage [16] and the rest is history.

In 1970, Bryn Bridges had established a small Medical Research Council-funded Unit, the MRC Cell Mutation Unit (CMU) on the University of Sussex campus. It was housed in a wood and glass shack (Fig. 2A), just across the way from the School of Biological Sciences, where I was working. One day in the summer of 1973 Bryn and Colin Arlett approached me after a seminar, told me that one of their four-man scientific staff was leaving and offered me the job. Recruitment was rather simpler in those days – no HR department, no obligatory advertising and interviewing. If you found someone you wanted to hire, you just hired them. Needless to say, I jumped at the opportunity and stayed at the CMU for the next 28 years – though we did graduate to a real brick building in 1980. 1973 was also a major milestone for us domestically with the birth of our son, Paul, to be followed a few years later by our daughter Anna.

2. XP variants, Cockayne syndrome and other repair-deficient disorders

My new colleague at the CMU, Colin Arlett, a mammalian cell biologist, was about to embark on studying the effects of DNA damaging agents on primary human fibroblasts, on which few people were working at that time. Jim Cleaver had discovered the defect in excision repair of UV damage in xeroderma pigmentosum (XP) in1968 [17], but in 1971, Jay Robbins and colleagues identified a XP patient with normal repair, which they designated as an XP variant [18], and Dirk Bootsma and colleagues identified another two of these XP variants [19]. Taking advantage of Colin's cell culture expertise, I decided to look if PRR was defective in XP variants. As it happens, we had just started what was to become an almost 40-years' collaboration with the DNA repair labs of Dirk Bootsma and then Jan Hoeijmakers in Rotterdam and of Paul Lohman followed by Leon Mullenders in Rijswijk and later in Leiden in the Netherlands. This collaboration blossomed over that period and was extended to include other major DNA repair labs in Europe. We were able to obtain European Union funding for collaborative projects over the next 35 years, as well as establishing the 5-yearly DNA repair meetings in the Netherlands – the most recent of which took place just last month (April, 2011). These collaborations played a major role in establishing and maintaining the prominent role of European labs in the DNA repair field.

To return to the XP-V story, Dirk Bootsma supplied us with their XP variant fibroblast strains and my technician, Sue Kirk-Bell, carried out many more sucrose gradient experiments, the sum of which did indeed indicate that XP-V cells were defective in PRR. Caffeine had been shown to sensitise cells to various DNA damaging agents and this had been extensively studied in rodent cells by Mike Rauth (e.g. [20]). Additionally I and others had shown that caffeine inhibited PRR in rodent cells [21]. Remarkably, we found that it had no effect on PRR in normal human fibroblasts, but completely inhibited the already deficient PRR in XPV fibroblasts, thus generating a huge difference between the normal and XPV cells [22]. In 1970s, effects of caffeine were interpreted in terms of its inhibitory effects on cyclic AMP phosphodiesterase, whereas these days everybody thinks of it as an inhibitor of ATR and ATM. I am pretty convinced that its effect on PRR is independent of both of these – it inhibits at low doses and its effect is immediate – and that it acts more directly.

Dirk Bootsma was not convinced by our results and decided to send us three samples without disclosing their origin. We had now optimised our protocol and were able immediately to identify them as being normal, XPV and excision-defective XP. The data from that blind experiment are shown in Fig. 4 of the paper that was published in PNAS in 1975 (see Fig 2B) [22] and is my most cited paper. We then built on this work and spent a lot of time in the next few years debating in the literature and at meetings whether the replication fork was blocked at lesions, whether it restarted beyond the lesion after a delay, or whether synthesis bypassed the lesion directly without discontinuities (e.g. [23]). These issues were not resolved and by 1980, studies on PRR ran out of steam and the whole field effectively went to sleep for almost 20 years. However these same questions are currently once again under investigation in several labs (e.g. [24–26]).

After discovering the defect in XP variant cells, we started to contact clinicians to obtain samples from patients with other disorders to see if they might have similar or other defects in DNA repair. Most of these led nowhere, but there were a few notable exceptions. In 1975 Bryn Bridges discussed our work with David Harnden, the Head of the Department of Cancer Studies at Birmingham University and David sent down a young colleague, Malcolm Taylor, with cells from three different disorders to look for possible DNA repair defects. One of these was ataxia-telangiectasia and together with Colin Arlett, Malcolm showed that they were sensitive to ionising radiation [27], a discovery that sparked off a whole new research area that led to the cloning of the ATM gene 20 years

later [28]. I was a little disappointed that we found no evidence for a defect in double-strand break repair using the relatively insensitive techniques that were available at the time [27,29].

In 1977, we were visited by Roy Schmickel, a paediatrician from Michigan, who introduced us to Cockayne syndrome. He informed us that he and Ernest Chu had shown that CS cells were sensitive to UV-irradiation, but had no defect in NER [30]. We confirmed these observations as well as showing no defect in PRR. I reasoned that if the cells were sensitive to UV using a colony-forming assay, which gave results 3 weeks after irradiating the cells, we must be able to find a defect earlier after irradiation. Being a great believer in simple experiments, I thought we could start off by simply measuring the rate of DNA synthesis by thymidine incorporation after irradiation. DNA synthesis was, as expected, inhibited by irradiation, but it recovered after a few hours in normal cells. In contrast, in CS cells the recovery did not happen [31]. We seemed to be onto something. We next showed that this could not be the primary defect as we could still observe cellular sensitivity to UV in quiescent cells that never divided after irradiation. So my first graduate student, Lynne Mayne, analysed RNA synthesis, again by simply measuring incorporation of uridine. Here the effect was even more dramatic than for DNA synthesis. RNA synthesis was inhibited but recovered very rapidly in normal cells, but again there was no recovery in CS cells. I cannot resist reproducing a few lines from the discussion of our 1982 paper [32]: "It is conceivable, however, that transcription-terminating lesions, i.e., those lesions in the transcribing regions of DNA, are potentially more lethal than the bulk of the lesions, and that these, comprising only a small fraction of the total damage (<10%), may be excised rapidly in normal cells by some special ER mechanism. On this model, the CS gene product would control this special ER pathway, but it would not be involved in overall ER". I was naturally pretty pleased with myself a few years later for having predicted transcription-coupled repair, which was discovered by Bohr, Hanawalt, Mellon and colleagues [33,34], and its defect in CS, which was shown by Mullenders and colleagues [35]. Equally naturally I would have been even more pleased if we had made these discoveries ourselves!

Around the mid 1980s, I realised that our simple incorporation tests could be used diagnostically to confirm or exclude clinical (and prenatal) diagnoses of XP and CS [36] and tried to persuade our paymasters at MRC to fund a technician to do this work. They were extremely reluctant as they felt that this was work that should be done by the National Health Service, not the Medical Research Council. We managed to persuade them that this work would generate valuable samples for research and after a long and depressing afternoon, they reluctantly agreed to underwrite the salary of the technician, but insisted that we recoup the money by charging clinicians for the tests. As it happened, this coincided with Margaret Thatcher's policy of marketisation of the Health Service, so although I despised almost everything that she stood for, her policy perhaps enabled our diagnostic service to be established. Over the intervening years, we have received samples from over 1000 patients from all over the world and have diagnosed over 110 cases of XP, 160 cases of CS and carried out 70 prenatal diagnoses for CS in affected families. In the meantime, MRC policy has changed and at our last quinquennial review, we were told that this was just the kind of translational work that MRC-funded scientists should be doing!!!

In 1982 David Webster, a clinical immunologist from London, sent to Colin Arlett a sample from an immunodeficient patient, whose cells we designated 46BR. Ian Teo, Colin's graduate student, found that unlike XP and A-T cells, which were sensitive to specific groups of genotoxins, 46BR seemed to be moderately sensitive to almost all of them [37]. This suggested to us that it might be deficient in a late step in repair processes, and indeed we found evidence for defects in DNA ligation [38] and together with the Lindahl group, we showed that 46BR had some features in common with Bloom syndrome [39]. A couple of years later Barnes and Lindahl cloned the human DNA ligase I gene [40] and looked for mutations in Bloom syndrome. They did not find any mutations in DNA ligase I in Bloom syndrome, but they did discover that 46BR was a compound heterozygote for mutations in DNA ligase I [41]. 46BR remains the only known patient with a defect in this protein and it has proven very useful in understanding the role of DNA ligase I in different processes.

3. DNA repair in *Schizosaccharomyces pombe* and the Smc5-6 complex

In 1985, our colleagues in Rotterdam cloned the first human NER gene (*ERCC1*) by transfection of a hamster UV-sensitive mutant [42] and went on to clone many more DNA repair genes using similar techniques. I have to admit that we were slow off the mark to harness recombinant DNA technology to our research, and although we did make some contributions in the mutagenesis field [43], they were not of major significance. In 1987 Melanie Lee and Paul Nurse cloned the human *CDC2* cDNA by its ability to complement the *S. pombe* cdc2 mutant [44]. I thought this might be a good way to clone human repair genes and had the good fortune to have some *S. pombe* experts close at hand. Furthermore a bunch of *S. pombe* "*rad*" mutants had been isolated in the mid 70s (reviewed in [45]) but very little had been done with them since that time. We thought it would be a good idea to clone a *S. pombe* repair gene first and Mike Fenech, who spent a year in my lab, together with Felicity Watts, Jo Murray and Tony Carr, succeeded in cloning the first *S. pombe* repair gene, rad4/cut5 [46]. We had the good fortune to be able to hire Tony Carr in the CMU in 1988 and within about 6 months Tony together with Jo Murray and Felicity Watts cloned about ten *S pombe* DNA repair genes and together, we spent the next few years characterising them and assigning them to different pathways. These included genes involved in classical NER [47,48], in a second repair pathway peculiar to *S. pombe* and a few other organisms [49], and in cell cycle checkpoints (e.g. [50]). Tony went on to study the latter in great depth and rapidly became a world leader in this area. In fact we never did clone any human genes by complementation of the *S. pombe* mutants and it is unlikely that this approach would have been successful except perhaps with one or two simple genes. We did however succeed in using degenerate PCR to clone the human homolog of *S. pombe rad2*, which turned out to be the nuclease subsequently named Fen1 [49].

One gene was left as a poor relation in our study of *S. pombe* repair genes and was at the time called *rad18* (though it is unrelated to *S. cerevisiae RAD18*). This gene was analysed gradually in the lab by a succession of summer students and visiting workers, and even on occasion by my own fair hand. In 1994, we had completed our analysis and sent a paper to Mol. Cell. Biol. on a protein involved in recombination repair with some leucine zipper motifs. It was rejected. At about the same time, several reports appeared in the literature, describing a new class of structural maintenance of chromosome (SMC) proteins, which were present in complexes involved in chromosome cohesion and condensation [51–53]. It became clear to us that our Rad18 protein was closely related to the SMC proteins, so we resubmitted the paper with little extra data to Mol. Cell. Biol., who now considered it extremely interesting and it was accepted in 1995 [54]. My lab has worked *inter alia* on this protein, now termed Smc6, for the last 15 years. We discovered its Smc partner protein Smc5 [55], as well as four other members of the Smc5-6 complex, Nse1-4 [56] and worked out how the various components fit together [56,57]. We also have identified and analysed the orthologous complex in human cells [58] and generated mice mutated in Smc6 (unpublished).

Alongside the *S. pombe* work, my interest in human disorders turned to trichothiodystrophy (TTD). Miria Stefanini had shown

that photosensitive TTD patients were defective in NER, like XP cells, and moreover were assigned to the XP-D complementation group [59]. We initiated a long-standing collaboration with Miria, extending her findings [60] and speculating and trying to understand how mutations in the *XPD* gene could result in such different phenotypes in XP and TTD. We still do not really know the complete answer to this, though there have of course been some major advances. The first was the cloning of the *ERCC2* gene by Christine Webber and Larry Thompson and demonstration that this was the *XPD* gene [61], the second was the seminal discovery by the group of Jean-Marc Egly in collaboration with the Rotterdam group that XPB and XPD were components of the transcription factor TFIIH, which had two functions, in NER and transcription [62]. Following on from these exciting findings we were able to identify the first mutations in the *XPD* gene, in TTD patients [63], and subsequently in many others and, importantly, we were able to show that each mutation site is disease-specific. R683W, found in the majority of XP patients in the XP-D group, has never been found in a TTD patient, whereas, R112H and R722W, fairly common in TTD, are never found in XP patients [64]. These findings were consistent with the idea proposed by several groups that XP is a repair syndrome, whereas TTD is a transcription syndrome.

4. Genome Damage and Stability Centre

The MRC CMU was established by Bryn Bridges and it is a bizarre policy of the MRC that when the Director of a so-called *ad personam* Unit retires, the Unit is closed. Bryn was due to retire in 2001 and MRC made it clear that the CMU would not continue. This left myself, Tony Carr and Penny Jeggo (who had joined us in 1989) in a very uncertain position – during 1996–1998, we did not know where we were going to go. We visited various university departments around the country but were not happy with anything that was on offer. This was a very unsettling period. Ultimately Tony Moore, Dean of Life Sciences and subsequently Deputy Vice-Chancellor at Sussex persuaded the university to provide us with a new building and three university-funded senior positions. For a small university like Sussex without any substantial financial assets, this was a major and risky commitment. MRC then agreed to fund the infrastructure of a joint University-MRC venture and the Genome Damage and Stability Centre (GDSC) was established in 2001 with myself as Chairman and Tony Carr as Director. In the following years we were able to hire Keith Caldecott, Aidan Doherty and more recently Jessica Downs to form a very strong team focussed on DNA repair and genome stability. We have been delighted with the success of the GDSC, which has doubled in size since its inception and now harbours 17 research groups.

5. PRR and TLS awake from a 20-year slumber

One of the few genes that had escaped identification in the 1980s and 1990s was the gene defective in XP variants, but the field of postreplication repair was beginning to wake up again around 1998. Robert Fuchs contacted me to tell me that his postdoc Agnes Cordonnier had developed a system for analysing TLS in human cell-free extracts and they were eager to analyse TLS in XP variant extracts. So we resurrected XPV cultures that had been in liquid nitrogen storage for 25 years and Agnes was indeed able to demonstrate a defect in TLS in XPV extracts [65]. Shortly afterwards, Roger Woodgate (who had done his PhD at Sussex with Bryn Bridges in 1980s), told me that he had found a gene related to Rad30 in yeast that was a good candidate for the XPV gene. We sequenced this gene in several XPV cell lines but did not find any mutations – the gene turned out to encode DNA polymerase ι [66], which is closely related to polymerase η, the real XPV gene that was identified by the Hanaoka and Prakash groups in June 1999 [67,68].

One week after the identification of the XPV-polη gene had been published, Patricia Kannouche joined my lab on a grant which was to clone the gene defective in XP-V cells! We quickly had to rethink the project and as Patricia had gained a lot of expertise on localisation studies, she decided to analyse the localisation of polη. This was a highly productive period and led to a series of publications on the localisation of all of the Y-family DNA polymerases, first polη [69], subsequently polι in collaboration with Roger Woodgate [70], Rev1with Robert Fuchs [71] and polκ with Tomoo Ogi [72].

Following on the discovery by Stefan Jentsch and colleagues that PCNA was ubiquitinated in *S. cerevisiae* when the replication fork encountered DNA damage [73], Patricia decided to look if something similar happened in human cells. She found that it did indeed and furthermore that ubiquitination of PCNA increased its affinity for polη, providing a nice model for switching from replicative to TLS polymerase [74]. The following year, I was contacted by Ivan Dikic from Frankfurt, who told me that his student Magda Bienko had discovered that the Y-family polymerases all bound to ubiquitin and that, with Kai Hoffmann from Cologne, they had discovered novel ubiquitin-binding domains in the Y family polymerases. This led to an exciting and productive collaboration, in which Cath Green in my lab was able to complement the biochemical work by showing the biological importance of the ubiquitin-binding domains in these polymerases [75].

The work on TLS over the last 12 years has been particularly exciting and I was very gratified last year to be elected as a Fellow of the Royal Society. I have always sought to collaborate rather than compete and my work has benefited hugely from many collaborations with other laboratories all over the world. These have been stimulating both scientifically and personally. It goes without saying that I am also indebted to the excellent postdocs, graduate students and technicians who have worked with me over the years.

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